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=> s entner-doudoroff pathway
L1          680 ENTNER-DOUDOROFF PATHWAY

=> s L-amino acid and production
L2         1213 L-AMINO ACID AND PRODUCTION

=> s l2 and l1
L3            3 L2 AND L1

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L3    ANSWER 1 OF 3  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI    Production of L-amino acid
      comprises culturing microorganism, which is Gram-negative bacterium having
      Entner-Doudoroff pathway and which has been
      modified so that specific activities are enhanced.
AN    2004-212658 [20]   WPIDS
AB    US2003219882 A UPAB: 20040324
      NOVELTY - Production of L-amino acid
      comprises culturing a microorganism having an ability to produce an
      L-amino acid in a medium. The microorganism is
      a Gram-negative bacterium having the Entner-Doudoroff
      pathway and which has been modified so that 6-phosphogluconate
      dehydratase activity or 2-keto-3-deoxy-6-phosphogluconate aldolase
```

activity, or activities of both are enhanced.
DETAILED DESCRIPTION - **Production** of **L-amino acid** comprises culturing a microorganism having an ability to produce an **L-amino acid** in a medium to produce and accumulate the **L-amino acid** in the medium and collecting the **L-amino acid** from the medium. The microorganism is a Gram-negative bacterium having the **Entner-Doudoroff pathway** and which has been modified so that 6-phosphogluconate dehydratase activity or 2-keto-3-deoxy-6-phosphogluconate aldolase activity, or activities of both are enhanced. The **L-amino acid** is **L-amino acid** produced by a biosynthetic pathway utilizing pyruvic acid as an intermediate.
USE - For producing an **L-amino acid**, e.g. L-glutamic acid (preferably), L-arginine, L-glutamine, L-proline, L-leucine, L-isoleucine, L-valine, or L-alanine (claimed).
ADVANTAGE - The method improves productivity of L-amino acids in bacteria from a viewpoint different from known techniques.
Dwg.0/2

ACCESSION NUMBER: 2004-212658 [20] WPIDS
DOC. NO. CPI: C2004-084256
TITLE: **Production** of **L-amino acid** comprises culturing microorganism, which is Gram-negative bacterium having **Entner-Doudoroff pathway** and which has been modified so that specific activities are enhanced.
DERWENT CLASS: B05 D16 E19
INVENTOR(S): ASANO, T; HARA, Y; IZUI, H; NAKAMATSU, T; WATANABE, Y
PATENT ASSIGNEE(S): (AJIN) AJINOMOTO CO INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2003219882	A1	20031127	(200420)*		12

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2003219882	A1	US 2003-396488	20030326

PRIORITY APPLN. INFO: US 2003-396488 20030326

L3 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI **Production** of **L-amino acid** comprises culturing microorganism, which is Gram-negative bacterium having **Entner-Doudoroff pathway** and which has been modified so that specific activities are enhanced;
gene overexpression enhancement for strain improvement and amino acid preparation
AN 2004-12299 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - **Production** of **L-amino acid** comprises culturing a microorganism having an ability to produce an **L-amino acid** in a medium. The microorganism is a Gram-negative bacterium having the **Entner-Doudoroff pathway** and which has been modified so that 6-phosphogluconate dehydratase activity or 2-keto-3-deoxy-6-phosphogluconate aldolase activity, or activities of both are enhanced.
DETAILED DESCRIPTION - **Production** of **L-amino acid** comprises culturing a microorganism having

an ability to produce an **L-amino acid** in a medium to produce and accumulate the **L-amino acid** in the medium and collecting the **L-amino acid** from the medium. The microorganism is a Gram-negative bacterium having the **Entner-Doudoroff pathway** and which has been modified so that 6-phosphogluconate dehydratase activity or 2-keto-3-deoxy-6-phosphogluconate aldolase activity, or activities of both are enhanced. The **L-amino acid** is **L-amino acid** produced by a biosynthetic pathway utilizing pyruvic acid as an intermediate.

USE - For producing an **L-amino acid**, e.g. L-glutamic acid (preferably), L-arginine, L-glutamine, L-proline, L-leucine, L-isoleucine, L-valine, or L-alanine (claimed).

ADVANTAGE - The method improves productivity of L-amino acids in bacteria from a viewpoint different from known techniques. (12 pages)

ACCESSION NUMBER: 2004-12299 BIOTECHDS
TITLE: **Production** of **L-amino acid** comprises culturing microorganism, which is Gram-negative bacterium having **Entner-Doudoroff pathway** and which has been modified so that specific activities are enhanced;
gene overexpression enhancement for strain improvement and amino acid preparation
AUTHOR: HARA Y; IZUI H; ASANO T; WATANABE Y; NAKAMATSU T
PATENT ASSIGNEE: AJINOMOTO CO INC
PATENT INFO: US 2003219882 27 Nov 2003
APPLICATION INFO: US 2003-396488 26 Mar 2003
PRIORITY INFO: US 2003-396488 26 Mar 2003; US 2003-396488 26 Mar 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-212658 [20]

L3 ANSWER 3 OF 3 BIOTECHDS
TI **Production** of L-amino acids in Gram-negative bacteria, useful particularly for making glutamic acid, by increasing activity of **Entner-Doudoroff pathway** enzymes;
involving Enterobacter agglomerans culture medium
AN 2004-00510 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - **Production** of L-amino acids (I) by culturing a Gram-negative bacterium that: (a) contains the **Entner-Doudoroff pathway**; and (b) has been modified so that activity of 6-phosphogluconate dehydratase (EDD) and/or 2-keto-3-deoxy-6-phosphogluconate aldolase (EDA) are increased, is new. (I) Is produced by a pathway that uses pyruvate as intermediate.

BIOTECHNOLOGY - Preferred Bacteria: These are enterobacteria, particularly an Enterobacter and specifically E. agglomerans ATCC 12287 or AJ 13355, 13356 or 13601. The activity of EDA and/or EDD is increased:(a) by increasing the copy number of the gene, by transformation with recombinant DNA; or (b) by modifying regulatory regions, especially use of a stronger promoter, so that gene expression is increased. Optionally: (a) activity of other genes involved in biosynthesis of (I) is also increased, especially citrate synthase, phosphoenolpyruvate carboxylase and glutamate dehydrogenase; or (b) activity of enzymes that divert intermediates away from synthesis of (I) are down regulated, especially alpha-ketoglutarate dehydrogenase or isocitrate lyase, particularly by mutation or gene disruption by homologous recombination. Preferred Process: This is **production** of L-Glu or of (I) that are produced by biosynthetic pathways in which Glu is an intermediate (Arg, Gln, Pro, Leu, Ile, Val or Ala). The modified bacteria are cultured at 20-45degreesC and pH 5-9.

USE - The method is specifically used to produce L-glutamic acid.

ADVANTAGE - Increasing activity of the specified enzymes reduces

flow of carbon into the pentose phosphate pathway and increases that into the **Entner-Doudoroff pathway**, so increases the **production** of (I) that are derived from pyruvate intermediate.

EXAMPLE - The strain Enterobacter agglomerans AJ13601: (a) has a disrupted gene for alpha-ketoglutarate dehydrogenase; and (b) includes a plasmid containing citrate synthase (CS), phosphoenolpyruvate carboxylase and glutamate dehydrogenase genes. It was transformed with (a) pMV-CB(asterisk) containing the promoter of the Escherichia coli CS gene and the coding region of the Brevibacterium lactofermentum CS gene or (b) pMV-CB(asterisk).ED, as (a) but including the E. coli genes for 6-phoshogluconate dehydratase (EDD) and/or 2-keto-3-deoxy-6-phosphogluconate aldolase (EDA). Both transformants were grown on nutrient medium of pH 6. In (a) L-glutamic acid yield was 30.4 g/l after 12 hours, and in (b) it was 36.8 g/l after 16 hours.(18 pages)

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ACCESSION NUMBER: 2004-00510  BIOTECHDS
TITLE:            Production of L-amino acids in Gram-negative
                  bacteria, useful particularly for making glutamic acid, by
                  increasing activity of Entner-Doudoroff
                  pathway enzymes;
                     involving Enterobacter agglomerans culture medium
AUTHOR:           HARA Y; IZUI H; ASANO T; WATANABE Y; NAKAMATSU T
PATENT ASSIGNEE:  AJINOMOTO CO INC
PATENT INFO:      EP 1352966 15 Oct 2003
APPLICATION INFO: EP 2003-6936 26 Mar 2003
PRIORITY INFO:    JP 2002-88668 27 Mar 2002; JP 2002-88668 27 Mar 2002
DOCUMENT TYPE:    Patent
LANGUAGE:         English
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     FILE 'MEDLINE, SCISEARCH, EMBASE, WPIDS, FSTA, JICST-EPLUS, BIOSIS,
     BIOTECHDS' ENTERED AT 13:57:54 ON 09 JAN 2006
L1          680 S ENTNER-DOUDOROFF PATHWAY
L2         1213 S L-AMINO ACID AND PRODUCTION
L3            3 S L2 AND L1

=> s l2 and methanol
L4           18 L2 AND METHANOL

=> d l4 ti abs ibib tot

L4   ANSWER 1 OF 18     MEDLINE on STN
```

TI Compared D-amino acid oxidase expression in different Pichia pastoris host strains.

AB To compare the DAAO expression level in different Pichia pastoris host strains, the gene encoding DAAO from Trigonopsis variabilis was cloned into plasmid pPIC3.5k and then transformed into P. pastoris GS115 and KM71 respectively. The positive transformants PDK13 (MutS) and PD27 (Mut+) were obtained by PCR analysis. Their optimal and different expression conditions were investigated. To compare with PD27, PDK13 was determined to poss a slower consumption of **methanol**, a longer induction time, a lower oxygen request and apparently higher expression of DAAO. The highest expression levels were reached up to 2700, 2500 IU/L in shaking flask and 10140, 8463.5 IU/L in fermentor respectively. The over-expression of DAAO can meet its large demand for **production** of 7-ACA, alpha-keto acid and **L-amino acid**. In addition, the phenylpyruvate and L-phenylalanine were obtained by crude DAAO reacting with DL-phenylalanine at 37 degrees C for 3h.

ACCESSION NUMBER: 2005320159 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15968991
TITLE: Compared D-amino acid oxidase expression in different Pichia pastoris host strains.
AUTHOR: Feng Mei-Qing; Huang Hai; Shi Xun-Long; Yu Zhi-Liang; Yuan Zhong-Yi; Zhou Pei
CORPORATE SOURCE: Fudan University, Shanghai 200032, China.
SOURCE: Sheng wu gong cheng xue bao = Chinese journal of biotechnology, (2004 Jul) 20 (4) 572-7.
Journal code: 9426463. ISSN: 1000-3061.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20050623
Last Updated on STN: 20051215

L4 ANSWER 2 OF 18 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI **Production** of a sol-gel layer on a substrate useful for preparing devices e.g. biosensor involves neutralizing an acidified sol suspension and electro-depositing it over an electrically conductive surface.
AN 2004-468317 [44] WPIDS
AB WO2004048603 A UPAB: 20040712
NOVELTY - **Production** (P1) of a sol-gel layer on a substrate, comprising at least partially neutralizing an acidified sol suspension, contacting an electrically conductive surface (s) with the neutralized suspension, and applying an electrical potential to (s) to form a layer of sol-gel on it, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:
(1) producing (P2) a biological assay device such as a microassay or biosensor, comprising electrodepositing a layer of sol-gel containing a biological material onto a substrate; and
(2) a biological assay device comprising (s) and a sol-gel containing at least one biological material.
USE - For producing a biological assay device (e.g. a biosensor or microarray) comprising 2 electrodes to detect analytes (claimed).
ADVANTAGE - The method allows microarrays and biosensors to be made inexpensively. The biosensors prepared by using this method have increased sensitivity (100-fold) than those prepared in prior arts. Stock solutions of acidified sol can be stored for long time. The enzymes may be added as and when a specific electrode needs to be produced. This allows greater flexibility in the **production** of microarrays and biosensors.
Dwg.0/3
ACCESSION NUMBER: 2004-468317 [44] WPIDS
DOC. NO. NON-CPI: N2004-370030
DOC. NO. CPI: C2004-175494
TITLE: **Production** of a sol-gel layer on a substrate useful for preparing devices e.g. biosensor involves neutralizing an acidified sol suspension and electro-depositing it over an electrically conductive surface.
DERWENT CLASS: A89 B04 D16 E11 J04 S03
INVENTOR(S): DALE, N E; DRONIOU, M; LLAUDET, E
PATENT ASSIGNEE(S): (UYWA-N) UNIV WARWICK
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2004048603	A2	20040610	(200444)*	EN	29

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE

LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP
KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG
PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ
VC VN YU ZA ZM ZW
AU 2003283590 A1 20040618 (200471)
EP 1565565 A2 20050824 (200556) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV
MC MK NL PT RO SE SI SK TR

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2004048603	A2	WO 2003-GB4984	20031118
AU 2003283590	A1	AU 2003-283590	20031118
EP 1565565	A2	EP 2003-775567	20031118
		WO 2003-GB4984	20031118

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2003283590	A1 Based on	WO 2004048603
EP 1565565	A2 Based on	WO 2004048603

PRIORITY APPLN. INFO: GB 2002-27424 20021125

L4 ANSWER 3 OF 18 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI Enzymatic peptide synthesis, by contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase to form a dipeptide, and contacting the dipeptide with a deacetylase to form peptide.
AN 2003-569047 [53] WPIDS
AB WO2003048189 A UPAB: 20030820
NOVELTY - Enzymatic peptide synthesis (M), comprising contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase (PL) so that PL catalyzes formation of peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making a dipeptide (DP), and contacting DP with a deacetylase (DA) so that DA catalyzes deacylation of DP, is new.

DETAILED DESCRIPTION - An enzymatic process (M) for synthesizing a peptide involves:

(a) providing at least two amino acids, at least one peptide and at least one amino acid, or at least two peptides;

(b) providing a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase are active under different reaction conditions and the peptide ligase is active after exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active;

(c) contacting the amino acids, or the peptide and amino acid, or peptides, with the peptide ligase under conditions, where the peptide ligase catalyzes the formation of a peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making at least a dipeptide; and

(d) contacting the peptide with the deacetylase under conditions, where the deacetylase catalyzes the deacylation of the peptide, to synthesize a peptide or a polypeptide.

Alternatively, the method comprises:

(a) providing reaction chamber comprising a peptide ligase and a deacetylase, where the peptide ligase and deacetylase are active under different reaction conditions, and the peptide ligase is active after

exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active;

(b) adding at least two amino acids, at least an amino acid and a peptide, or at least two peptides to the reaction chamber under conditions, where the peptide ligase is active and the peptide ligase catalyzes the formation of a peptide bond between the amino acids, or between the peptide and the amino acid, or between the peptides; and

(c) changing the conditions in the reaction chamber to conditions, where the deacetylase is active and the deacetylase catalyzes the deacylation of the peptide formed.

An INDEPENDENT CLAIM is also included for a product of manufacture (I) comprising a reaction chamber for synthesizing a peptide comprising a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase are active under different reaction conditions and the peptide ligase is active after exposure to reaction conditions, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active.

USE - (M) is useful for synthesizing a peptide or polypeptide, where the peptide or polypeptide is between 2-50, preferably 5-25 peptides in length. The peptide is a D-amino acid, a naturally occurring **L-amino acid**, or a glycosylated, phosphorylated or non-naturally occurring amino acid, where the non-naturally occurring amino acid is a non-naturally occurring aromatic amino acid comprising a D- or L-naphthylalanine, D- or L-phenylglycine, D- or L-2 thieneylalanine, D- or L-1, -2, 3- or 4-pyreneylalanine, D- or L-3 thienylalanine, D- or L-(2-pyridinyl)-alanine, D- or L-(3-pyridinyl)-alanine, a D- or L-(2-pyrazinyl)-alanine, D- or L-(3-pyridinyl)-alanine, D- or L-(2-pyrazinyl)-alanine, D- or L-(4-isopropyl)-phenylglycine, a D-(trifluoromethyl)-phenylglycine, a D-(trifluoromethyl)-phenylalanine, a D-p-fluoro-phenylalanine, a D-or L-p-biphenylphenylalanine, a D-or L-p-methoxy-biphenylphenylalanine, D- or L-2-indole(alkyl)alanines. The non-naturally occurring aromatic amino acid comprises a thiazolyl, a thiophenyl, a pyrazolyl, a benzimidazolyl, a naphthyl, a furanyl, pyrrolyl, or pyridyl aromatic ring. The non-naturally occurring amino acid comprises a D- or L-alkylalanine. The alkyl of the alkylalanines comprises a substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl or iso-pentyl (claimed). (I) is useful for synthesizing commercial drugs such as Lupron (RTM), Leuplin (RTM), Miacalcin (RTM), Zoladex (RTM), Sandostatin (RTM), Decapeptyl (RTM), DDAVP (RTM), Glucagon (RTM), Intergrilin (RTM), Suprefact (RTM), Stilamin (RTM), Synarel (RTM), Zadaxin (RTM) or Copaxone (RTM).

ADVANTAGE - (M) uses mild conditions for coupling and deprotection steps, has a reduced requirement for organic solvents, avoids expensive and toxic coupling reagents, reduces the **production** of toxic byproducts, and allows for biocatalyst recycling.

Dwg.0/6

ACCESSION NUMBER: 2003-569047 [53] WPIDS
DOC. NO. CPI: C2003-153465
TITLE: Enzymatic peptide synthesis, by contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase to form a dipeptide, and contacting the dipeptide with a deacetylase to form peptide.
DERWENT CLASS: B04 D16
INVENTOR(S): BURK, M J; DESANTIS, G; BURK, M; DE SANTIS, G
PATENT ASSIGNEE(S): (DIVE-N) DIVERSA CORP
COUNTRY COUNT: 102
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003048189	A2	20030612	(200353)*	EN	43

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU

MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
ZM ZW
US 2003143664 A1 20030731 (200354)
AU 2002364710 A1 20030617 (200419)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003048189	A2	WO 2002-US38588	20021203
US 2003143664	A1 Provisional	US 2001-336580P	20011203
		US 2002-309758	20021203
AU 2002364710	A1	AU 2002-364710	20021203

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2002364710	A1 Based on	WO 2003048189

PRIORITY APPLN. INFO: US 2001-336580P 20011203; US 2002-309758 20021203

L4 ANSWER 4 OF 18 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI Efficient **production** of L-amino-acids e.g. L-lysine by fermenting transformed Methylophilus bacterium with enhanced dihydrodipicolinate synthase and aspartokinase activities or casamino acid requirement using **methanol**.
AN 2000-672679 [65] WPIDS
AB WO 200061723 A UPAB: 20001214
NOVELTY - A bacterium of Methylophilus genus can produce an **L-amino-acid** efficiently, which is a transformed bacterium with enhanced enzyme activity.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a process for preparing an **L-amino-acid** by culturing Methylophilus for accumulation of the product in culture medium before isolation;

(2) a DNA having a 1981 base pair (bp) sequence, encoding a 409 amino acid sequence, having aspartokinase activity;

(3) a DNA having a 1452 bp sequence, encoding a 370 amino acid sequence, aspartic semi-aldehyde dehydrogenase activity;

(4) a DNA having a 3098 bp sequence, encoding a 296 amino acid sequence, having dihydrodipicolinate synthase activity;

(5) a DNA having a 3390 bp sequence, encoding the 286 amino acid sequence, dihydrodipicolinate reductase activity; and

(6) a DNA having the 2566 bp sequence, encoding the 415 amino acid sequence, having diaminopimelate decarboxylate activity.

All sequences are given in the specification.

USE - The method is for the **production** of L-amino-acids including L-lysine, L-valine, L-leucine, L-isoleucine or L-threonine (claimed).

ADVANTAGE - The process is efficient and the bacterium used has enhanced enzyme activity..

DESCRIPTION OF DRAWING(S) - Construction of mutated dapA-containing plasmid RSF24P.

Dwg.1/7

ACCESSION NUMBER: 2000-672679 [65] WPIDS
DOC. NO. CPI: C2000-203771

TITLE: Efficient **production** of L-amino-acids e.g. L-lysine by fermenting transformed Methylophilus bacterium with enhanced dihydrodipicolinate synthase and aspartokinase activities or casamino acid requirement using **methanol**.
DERWENT CLASS: B05 D16 E19
INVENTOR(S): GUNJI, Y; MIYATA, Y; OBA, M; SHIMAOKA, M; SUGIMOTO, S; TSUJIMOTO, N; YASUEDA, H
PATENT ASSIGNEE(S): (AJIN) AJINOMOTO CO INC
COUNTRY COUNT: 35
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000061723	A1	20001019	(200065)*	JA	92
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU BR CA CN CZ HU ID JP KR MX PL RU SK US VN ZA					
AU 2000036745	A	20001114	(200108)		
BR 2000009550	A	20020205	(200213)		
EP 1188822	A1	20020320	(200227)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
CN 1346402	A	20020424	(200251)		
JP 2000611648	X	20020723	(200263)		
MX 2001010151	A1	20030701	(200366)		
RU 2250266	C2	20050420	(200528)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000061723	A1	WO 2000-JP2295	20000407
AU 2000036745	A	AU 2000-36745	20000407
BR 2000009550	A	BR 2000-9550	20000407
		WO 2000-JP2295	20000407
EP 1188822	A1	EP 2000-915436	20000407
		WO 2000-JP2295	20000407
CN 1346402	A	CN 2000-806019	20000407
JP 2000611648	X	JP 2000-611648	20000407
		WO 2000-JP2295	20000407
MX 2001010151	A1	WO 2000-JP2295	20000407
		MX 2001-10151	20011008
RU 2250266	C2	WO 2000-JP2295	20000407
		RU 2001-130146	20000407

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000036745	A	Based on	WO 2000061723
BR 2000009550	A	Based on	WO 2000061723
EP 1188822	A1	Based on	WO 2000061723
JP 2000611648	X	Based on	WO 2000061723
MX 2001010151	A1	Based on	WO 2000061723
RU 2250266	C2	Based on	WO 2000061723

PRIORITY APPLN. INFO: JP 1999-368097 19991224; JP 1999-103143 19990409; JP 1999-169447 19990616

L4 ANSWER 5 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Novel modified microorganism capable of showing enhanced expression of ybjE gene and producing **L-amino acid** such as L-lysine, L-arginine, L-ornithine, useful as additives for animal

feed, amino acid infusions and precursor of sweeteners;
amino acid **production** via genetically engineered bacterium culture for use in food

AN 2005-25581 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A microorganism (I) having an **L-amino acid**-producing ability, where the microorganism is modified so that expression of ybjE gene is enhanced, is new.

BIOTECHNOLOGY - Preferred Microorganism: In (I), the expression of the ybjE gene has been enhanced by increasing a copy number of the ybjE gene, or by modifying an expression regulatory sequence of ybjE gene. The amino acid sequence of a protein encoded by ybjE gene is chosen from fully defined 315 amino acid (SEQ ID NO: 2), 299 amino acid (SEQ ID No: 9) and 298 amino acid (SEQ ID No: 10) sequences given in the specification, where the protein has an **L-amino acid**-export ability. The ybjE gene is chosen from a DNA comprising a fully defined 948 nucleotides (SEQ ID NO: 1) sequences given in the specification, and a DNA hybridizable under stringent conditions with a nucleotide sequence of SEQ ID No: 1 or a probe that can be prepared from the nucleotide sequence of SEQ ID No: 1, where the DNA encodes a protein having an **L-amino acid** -export ability. The ybjE gene is chosen from a DNA having a nucleotide sequence of nucleotide numbers 49-948 in SEQ ID No: 1, and a DNA hybridizable under stringent conditions with a nucleotide sequence of nucleotide numbers 49-948 in SEQ ID No: 1 or a probe that can be prepared from the nucleotide sequence of nucleotide numbers 49-948 in SEQ ID No: 1, and where the DNA encodes a protein having an **L-amino acid**-export ability. The **L-amino acid**-export ability of the microorganism is increased by enhancing expression of ybjE gene. The resistance of the microorganism to an **L-amino acid** or **L-amino acid** analog is increased by enhancing expression of the ybjE gene. The microorganism belongs to an Enterobacteriaceae family. The microorganism belonging to an Enterobacteriaceae family is a microorganism belonging to the genus Escherichia. The microorganism is a Coryneform bacterium or a **methanol**-assimilating bacterium. The **methanol** -assimilating bacterium is a microorganism belonging to the genus Methylophilus or Methylobacillus.

USE - (I) is useful for producing **L-amino acid**, which involves culturing (I) in a medium to produce and cause accumulation of the **L-amino acid**, and collecting the **L-amino acid** from the medium or (I), or by culturing (I) in a liquid containing **methanol** as a major carbon source to produce and cause the accumulation of **L -amino acid** and collecting **L-amino acid** from (I). The **L-amino acid** is chosen from L-lysine, L-arginine, L-ornithine, L-histidine, L-isoleucine, L-threonine, L-proline, L-phenylalanine, L-cysteine, and L-glutamic acid (claimed). The L-lysine, L-isoleucine, L-threonine, L-proline are useful as additives for animal feed, components of health food and amino acid infusions. The L-arginine and L-ornithine are useful as liver function-promoting agents, amino acid infusions and components of comprehensive amino acid preparations. The L-histidine is useful as a liver function-promoting agent and as a precursor of histamine. The L-phenylalanine is useful as a precursor of sweeteners.

ADVANTAGE - (M1) is cost effective and efficient, and introduction of ybjE gene increases **L-amino acid** export ability.

EXAMPLE - The AS1 strains introduced with pRSybjEdapA, pRSybjE, pRSdaPA, or pRS, were plated on SEII plate containing 20 mg/l of streptomycin and cultured overnight at 37degreesC. Then, the cells from 0.3 cm2 of the medium surface were scraped, inoculated in to SEII

production medium (20 ml) containing streptomycin (20 mg/l), and cultured at 37degreesC for 34 hours with shaking. After completion of the culture, the cells were removed by centrifugation, and the L-lysine concentration in the culture supernatant was determined by using an amino acid analyzer. The strain that had been introduced with pRSybjEdapA showed increased L-lysine accumulation (1.38 g/l) compared to the strains introduced only with pRSdapA (0.12 g/l) or pRsybje (0.7 g/l), and pRS (0 g/l) . Thus, it was found that enhancing both the ybjE gene and the dapAasterisk gene had a synergistic effect on L-lysine **production** .(85 pages)

ACCESSION NUMBER: 2005-25581 BIOTECHDS
TITLE: Novel modified microorganism capable of showing enhanced expression of ybjE gene and producing **L-amino acid** such as L-lysine, L-arginine, L-ornithine, useful as additives for animal feed, amino acid infusions and precursor of sweeteners;
amino acid **production** via genetically engineered bacterium culture for use in food
AUTHOR: UEDA T; NAKAI Y; GUNJI Y; TAKIKAWA R; JOE Y
PATENT ASSIGNEE: AJINOMOTO CO INC
PATENT INFO: WO 2005073390 11 Aug 2005
APPLICATION INFO: WO 2005-JP1650 28 Jan 2005
PRIORITY INFO: JP 2004-23347 30 Jan 2004; JP 2004-23347 30 Jan 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-604908 [62]

L4 ANSWER 6 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI **Production** of target substance, e.g. L-amino acids, using coryneform bacterium, comprises culturing coryneform bacterium having ability to produce target substance in medium, and collecting target substance from medium or cells of bacterium;
L-amino acid production via modified bacterium culture
AN 2004-21654 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Target substance, e.g. L-amino acids, is produced using coryneform bacterium by culturing a coryneform bacterium having an ability to produce the target substance in a medium to result in accumulation of the target substance in the medium or cells of the bacterium, and collecting the target substance from the medium or cells of bacterium.

DETAILED DESCRIPTION - **Production** of target substance, e.g. L-amino acids, using coryneform bacterium comprises culturing a coryneform bacterium having an ability to produce the target substance in a medium to result in accumulation of the target substance in the medium or cells of the bacterium, and collecting the target substance from the medium or cells of bacterium. A **methanol** dehydrogenase, hexulose phosphate synthase gene, and phosphohexuloisomerase gene are introduced into the coryneform bacterium. The bacterium is modified so that an ability to utilize **methanol** is imparted. It contains **methanol** as carbon source.

BIOTECHNOLOGY - Preferred Process: The bacterium is further introduced with a gene encoding a **methanol** dehydrogenase activity-promoting factor. Preferred Component: The bacterium belongs to the genus corynebacterium, preferably Corynebacterium glutamicum.

USE - For producing a target substance, e.g. L-amino acids from L-lysine (claimed), nucleic acids, antibiotics, vitamins, growth factors, and physiologically actives substances, using coryneform bacterium.

ADVANTAGE - The invention enhances the ability of the bacterium to utilize **methanol**. (28 pages)

ACCESSION NUMBER: 2004-21654 BIOTECHDS
TITLE: **Production** of target substance, e.g. L-amino acids,

using coryneform bacterium, comprises culturing coryneform bacterium having ability to produce target substance in medium, and collecting target substance from medium or cells of bacterium;
L-amino acid
production via modified bacterium culture

AUTHOR: TAKESHITA R; YASUEDA H
PATENT ASSIGNEE: AJINOMOTO CO INC
PATENT INFO: EP 1454991 8 Sep 2004
APPLICATION INFO: EP 2004-5129 4 Mar 2004
PRIORITY INFO: JP 2003-57171 4 Mar 2003; JP 2003-57171 4 Mar 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-627851 [61]

L4 ANSWER 7 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI L-Lysine preparation with high productivity, by culturing **methanol**-utilizing bacteria auxotrophic for L-methionine, preferably new Methylophilus strains;
L-amino acid preparation by bacterium fermentation

AN 2004-20239 BIOTECHDS

AB DERWENT ABSTRACT:
NOVELTY - **Production** of L-lysine (I) involves culturing bacteria which utilize **methanol**, require L-methionine (II) for growth and produce (I) in a medium containing **methanol** as main carbon source, then recovering the (I) accumulated in the culture, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for Methylophilus bacteria which require (II) for growth and produce (I).

BIOTECHNOLOGY - Preferred Bacteria: The bacteria are of genus Methylophilus, preferably Methylophilus methylotrophus strains, especially Methylophilus strains modified to stimulate diaminopimelate enzymatic activity and the (I) secretion system. **Production**: The new Methylophilus bacteria requiring (II) for growth are obtained by mutation of wild strains using physical stimuli (e.g. UV-, X- or gamma-rays) or preferably a chemical mutagen (e.g. N-methyl-N'-nitro-N-nitroso-guanidine); or by using genetic engineering methods to suppress the activity of an enzyme involved in the synthesis of (II), e.g. by cleaving the gene metA (encoding homoserine o-acetyltransferase) in Methylophilus methylotrophus.

USE - For producing L-lysine (claimed).

ADVANTAGE - (I) are obtained with high efficacy from bacteria utilizing (inexpensive) **methanol** as carbon source. Conferring auxotrophy for (II) on **methanol**-utilizing bacteria significantly increases the amount of free (I) accumulated in the cells relative to the total amount of cell proteins, e.g. by a factor of 1.5 or more.

EXAMPLE - The wild strain Methylophilus methylotrophus AS1 (NCIMB 10515) was subjected to cleavage of the gene metA (encoding homoserine o-acetyltransferase). A DNA region of high homology with this gene (cleaved from Mycobacterium tuberculosis strain H37Rv (CAA17113)), chromosomal DNA from strain AS1 and plasmid pKD4 (encoding kanamycin resistance) were separately amplified by PCR, mixed and subjected to further PCR to give a 4.2 kb fragment, consisting of gene metA with the kanamycin resistance gene inserted. This fragment was introduced into strain AS1 by electroporation to give a strain auxotrophic for L-methionine (II), designated strain MR701. The L-lysine (I)-producing plasmid pSEA10 was introduced into strain MR701 by electroporation to give the (I)-producing strain MR701(pSEA10). Cells of this strain were used to inoculate **production** medium SEII containing 0.075 g/l (II), followed by culture under stirring at 37degreesC for 48 hours. The amount of (I) accumulated in the supernatant was 1.52 g/l, compared with

0.97 g/l using the strain AS1(pSEA12).(43 pages)

ACCESSION NUMBER: 2004-20239 BIOTECHDS

TITLE: L-Lysine preparation with high productivity, by culturing **methanol**-utilizing bacteria auxotrophic for L-methionine, preferably new Methylophilus strains; **L-amino acid** preparation by bacterium fermentation

PATENT ASSIGNEE: AJINOMOTO CO INC

PATENT INFO: FR 2850394 30 Jul 2004

APPLICATION INFO: FR 2004-816 29 Jan 2004

PRIORITY INFO: JP 2003-20513 29 Jan 2003; JP 2003-20513 29 Jan 2003

DOCUMENT TYPE: Patent

LANGUAGE: French

OTHER SOURCE: WPI: 2004-563572 [55]

L4 ANSWER 8 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI **Production** of an **L-amino acid** using an amino acid, an ammonium salt, a reducing coenzyme, and a microorganism with transaminase and amino acid dehydrogenase activity, to convert an alpha-keto acid into an **L-amino acid**; L-t-leucine preparation by bacterium fermentation and enzyme-catalyzed conversion

AN 2004-11885 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - **Production** (M1) of an **L-amino acid** (X) using an amino acid as an amino donor, an ammonium salt and coenzyme which has reducing power, and converting an alpha-keto acid or its salt into L-amino acids in the presence of a microorganism (or its product) which has transaminase activity and amino acid dehydrogenase activity.

BIOTECHNOLOGY - Preferred Method: The co-enzyme is reduced nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide phosphoric acid or their salts. Amino acid (X) is a compound of formula (I). R = alkyl group of two or more carbon atoms, a benzyl group, a p-hydroxy benzyl group or an **L-amino acid**. Preferred Microorganism: The microorganism providing the enzyme activity is preferably Brevibacterium ammoniagenes.

USE - Process M1 is useful for producing (X) such as L-t-leucine from alpha-keto acids (claimed).

ADVANTAGE - Process M1 enables **production** of large amounts of **L-amino acid**.

EXAMPLE - Microbial cells e.g. Brevibacterium ammoniagenes ATCC 6872, with transaminase activity and amino acid dehydrogenase activity, were inoculated into culture media comprising glucose, dipotassium hydrogen phosphate, magnesium sulfate, peptone, sodium chloride and yeast extract, and cultivated by shaking at 37 degrees C until the absorbance of 600 nm was 0.7. The cultivated microbial cells were collected and washed by 0.85% sodium chloride and then allowed to dry overnight. Dry microbial cells (10 mg/ml) were added to reaction solution (A) which contained 60 g/l of glutamic acid with respect to 40 g/l of trimethyl pyruvic acid, reaction solution (B) which contained 40 g/l of ammonium chloride, 100 g/l of glucose, 1.2 g/l of nicotinamide adenine dinucleotide (NADH), 1.2 g/l of nicotinamide adenine dinucleotide phosphoric acid (NADPH) and 0.41 g/l of glucose dehydrogenase, and reaction solution (C) which contained 60 g/l of glutamic acid, 40 g/l of ammonium chloride, 100 g/l of glucose, 1.2 g of NADH, 1.2 g of NADPH and 0.41 g/l of glucose dehydrogenase. The solutions were allowed to react at 30 degrees C for six days. To 100 microl of obtained reaction solution, 10 mM gamma amino butyric acid was added and extracted by 200 microl of 1-butanol. To 10 microl of obtained solution, 1% trimethylamine aqueous solution, 20 microl of acetonitrile and 0.2% of 50 microl of 2,3,4,6-tetra-o-acetyl-beta-D-glucoisothiocyanate (GITC)-acetonitrile

were added and allowed to react for 15 minutes at room temperature with respect to 20 microl of 1-butanol layers. 5 microl of the obtained solution was subjected to high performance layer chromatography which uses shim-pack CLC-ODS(M) column, and 60% aqueous solution of **methanol** as eluting solvent for analysis of L-t-leucine in a sample. The concentration of L-t-leucine in a sample was calculated based on the standard curve. The results showed that reaction solution (C) which had both the transaminase activity and amino acid dehydrogenase activity produced more amount of L-t-leucine when compared to reaction solution (A) having transaminase activity and reaction solution (B) having amino acid dehydrogenase activity. The concentration of L-t-leucine produced by using reaction solution (C) was found to be 3.4 g/l.(6 pages)

ACCESSION NUMBER: 2004-11885 BIOTECHDS
TITLE: **Production** of an **L-amino acid** using an amino acid, an ammonium salt, a reducing coenzyme, and a microorganism with transaminase and amino acid dehydrogenase activity, to convert an alpha-keto acid into an **L-amino acid**;
L-t-leucine preparation by bacterium fermentation and enzyme-catalyzed conversion
PATENT ASSIGNEE: TORAY IND INC
PATENT INFO: JP 2003284584 7 Oct 2003
APPLICATION INFO: JP 2002-94838 29 Mar 2002
PRIORITY INFO: JP 2002-94838 29 Mar 2002; JP 2002-94838 29 Mar 2002
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2004-172965 [17]

L4 ANSWER 9 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Enzymatic peptide synthesis, by contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase to form a dipeptide, and contacting the dipeptide with a deacetylase to form peptide;
enzyme-catalyzed peptide synthesis for use in the drug industry
AN 2003-20758 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Enzymatic peptide synthesis (M), comprising contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase (PL) so that PL catalyzes formation of peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making a dipeptide (DP), and contacting DP with a deacetylase (DA) so that DA catalyzes deacylation of DP, is new.

DETAILED DESCRIPTION - An enzymatic process (M) for synthesizing a peptide involves: (a) providing at least two amino acids, at least one peptide and at least one amino acid, or at least two peptides; (b) providing a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase are active under different reaction conditions and the peptide ligase is active after exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active; (c) contacting the amino acids, or the peptide and amino acid, or peptides, with the peptide ligase under conditions, where the peptide ligase catalyzes the formation of a peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making at least a dipeptide; and (d) contacting the peptide with the deacetylase under conditions, where the deacetylase catalyzes the deacylation of the peptide, to synthesize a peptide or a polypeptide. Alternatively, the method comprises: (a) providing reaction chamber comprising a peptide ligase and a deacetylase, where the peptide ligase and deacetylase are active under different reaction conditions, and the peptide ligase is active after exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction

conditions where the peptide ligase is active; (b) adding at least two amino acids, at least an amino acid and a peptide, or at least two peptides to the reaction chamber under conditions, where the peptide ligase is active and the peptide ligase catalyzes the formation of a peptide bond between the amino acids, or between the peptide and the amino acid, or between the peptides; and (c) changing the conditions in the reaction chamber to conditions, where the deacetylase is active and the deacetylase catalyzes the deacylation of the peptide formed. An INDEPENDENT CLAIM is also included for a product of manufacture (I) comprising a reaction chamber for synthesizing a peptide comprising a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase are active under different reaction conditions and the peptide ligase is active after exposure to reaction conditions, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active.

BIOTECHNOLOGY - Preferred Method: (M) further comprises changing conditions in the reaction chamber to conditions, where the peptide ligase is active and the deacetylase is inactive and adding at least one additional amino acid or peptide to the reaction chamber. (M) further comprises changing conditions in the reaction chamber to conditions so that the deacetylase is active. The temperature conditions are changed in the reaction chamber. (M) further comprises reiterating the process, thus making a longer peptide or polypeptide. The peptide ligase and the deacetylase are active at different temperature conditions. The reiterated process comprises thermocycling the peptide ligase and the deacylation reactions. The reaction chamber comprises a thermocycled bioreactor. The peptide ligase and the deacetylase are active at different pH conditions, at different salt conditions or at different solute conditions. The reaction chamber comprises a capillary array such as GIGAMATRIX (RTM). The peptide ligase and the deacetylase are immobilized. The peptide ligase is active at higher temperature than the deacetylase. The deacetylase is inactive and thermotolerant in the conditions set for the peptide ligase activity. The amino acids, or the peptide and the amino acid, are contacted with the peptide ligase under conditions comprising about a temperature of 50 degreesC. The deacylation reaction conditions comprise a temperature of 20 degreesC. The peptide ligase and the deacetylase reactions are thermocycled between 50-20 degreesC. The peptide ligase is a hydrolase (e.g. serine hydrolase), esterase or lipase, muramyl peptide synthetase, or a catalytic antibody. The deacetylase is an aminoacylase, D-aminoacylase, L-aminoacylase or a catalytic antibody. (M) further comprises use of at least two peptide ligases or at least two aminoacylases. The reaction is driven in favor of peptide catalysis and reducing aminolysis by reacting the peptide ligase under conditions comprising a low water environment, an organic solvent, or substantially pure organic solvent, or under conditions comprising removing the product upon its formation by precipitation or by liquid-liquid extraction. The reaction is driven in favor of peptide catalysis and reducing aminolysis by reacting the peptide ligase in a water and ethanol solvent, or in a pure **methanol** solvent or a pure ethanol solvent. (M) further comprises the injection of fresh enzyme into the reaction after each reiterated cycle. (M) further comprises the injection of fresh peptide ligase or deacetylase into the reaction. (M) comprises providing reaction chamber comprising a peptide ligase and a deacetylase, where the peptide ligase and deacetylase are active at different temperatures and the peptide ligase is active after exposure to the deacetylase's activity temperature and the deacetylase is active after exposure to the peptide ligase's activity temperature, and at least two amino acids, at least an amino acid and a peptide, or at least two peptides, reacting the reaction chamber under conditions where the peptide ligase is active and the deacetylase is inactive and the peptide ligase catalyzes the formation of a peptide bond between the amino acids, or between the peptide and the amino acid, or between the peptides, and changing the conditions in the reaction chamber to conditions where the

deacetylase is active and the peptide ligase is inactive and the deacetylase catalyzes the deacylation of the peptide formed. Preferred Product: In (I), the reaction chamber comprises a thermocycler. The reaction chamber is operably linked to an high pressure liquid chromatograph, mass spectrograph, a liquid chromatograph (LC), or a multiplex interfaced liquid chromatograph (LC)-mass spectrograph (MS)(LC-MS) system. The reaction chamber further comprises a desorption/ionization device. (I) further comprises an input for injection of enzyme or starting material into the reaction chamber, robotic arms to move microtiter plates between different platform components, temperature and humidity controlled incubators, liquid handling devices, bar-coding devices or plate readers. (I) enables high throughput robotic assays.

USE - (M) is useful for synthesizing a peptide or polypeptide, where the peptide or polypeptide is between 2-50, preferably 5-25 peptides in length. The peptide is a D-amino acid, a naturally occurring **L-amino acid**, or a glycosylated, phosphorylated or non-naturally occurring amino acid, where the non-naturally occurring amino acid is a non-naturally occurring aromatic amino acid comprising a D- or L-naphthylalanine, D- or L-phenylglycine, D- or L-2 thieneylalanine, D- or L-1, -2, 3- or 4-pyreneylalanine, D- or L-3 thienylalanine, D- or L-(2-pyridinyl)-alanine, D- or L-(3-pyridinyl)-alanine, a D- or L-(2-pyrazinyl)-alanine, D- or L-(3-pyridinyl)-alanine, D- or L-(2-pyrazinyl)-alanine, D- or L-(4-isopropyl)-phenylglycine, a D-(trifluoromethyl)-phenylglycine, a D-(trifluoromethyl)-phenylalanine, a D-p-fluoro-phenylalanine, a D-or L-p-biphenylphenylalanine, a D-or L-p-methoxy-biphenylphenylalanine, D- or L-2-indole(alkyl)alanines. The non-naturally occurring aromatic amino acid comprises a thiazolyl, a thiophenyl, a pyrazolyl, a benzimidazolyl, a naphthyl, a furanyl, pyrrolyl, or pyridyl aromatic ring. The non-naturally occurring amino acid comprises a D- or L-alkylalanine. The alkyl of the alkylalanines comprises a substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl or iso-pentyl (claimed). (I) is useful for synthesizing commercial drugs such as Lupron (RTM), Leuplin (RTM), Miacalcin (RTM), Zoladex (RTM), Sandostatin (RTM), Decapeptyl (RTM), DDAVP (RTM), Glucagon (RTM), Intergrilin (RTM), Suprefact (RTM), Stilamin (RTM), Synarel (RTM), Zadaxin (RTM) or Copaxone (RTM).

ADVANTAGE - (M) uses mild conditions for coupling and deprotection steps, has a reduced requirement for organic solvents, avoids expensive and toxic coupling reagents, reduces the **production** of toxic byproducts, and allows for biocatalyst recycling. (43 pages)

ACCESSION NUMBER: 2003-20758 BIOTECHDS
TITLE: Enzymatic peptide synthesis, by contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase to form a dipeptide, and contacting the dipeptide with a deacetylase to form peptide;
enzyme-catalyzed peptide synthesis for use in the drug industry
AUTHOR: DE SANTIS G; BURK M
PATENT ASSIGNEE: DIVERSA CORP
PATENT INFO: WO 2003048189 12 Jun 2003
APPLICATION INFO: WO 2002-US38588 3 Dec 2002
PRIORITY INFO: US 2001-336580 3 Dec 2001; US 2001-336580 3 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-569047 [53]

L4 ANSWER 10 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Novel DNA encoding variant of LysE protein from a coryneform bacterium, when introduced into **methanol** assimilating bacterium, facilitates excretion of L-lysine and/or L-arginine to outside of a cell;
plasmid pRSlysE-mediated lysE gene transfer to Methylophilus sp. for

amino acid **production**

AN 2003-11377 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - DNA (I) encoding variant of protein with loop region and six hydrophobic helixes facilitates excretion of L-lysine and/or L-arginine to outside of cell of a **methanol** assimilating bacterium when (I) is introduced into the bacterium, is new.

DETAILED DESCRIPTION - DNA (I) encoding variant of protein with loop region and six hydrophobic helixes and involved in excretion of L-lysine to outside of cell, where (I) encodes a mutant protein not containing loop region that is contained in wild-type protein and facilitates excretion of L-lysine, L-arginine or both of these L-amino acids to outside of a cell of a **methanol** assimilating bacterium when (I) is introduced into the bacterium, is new. INDEPENDENT CLAIMS are also included for: (1) A DNA encoding: (a) a protein which comprises a fully defined sequence of 124 amino acids (S1) as given in the specification; or (b) a protein which comprises a sequence of (S1) including substitution, deletion, insertion or addition of one or several amino acid residues and shows an activity for facilitating excretion of L-lysine, L-arginine or both of these L-amino acids to outside of a cell of **methanol** assimilating bacterium; (2) A Methylophilus bacterium (II), into which (I) or (II) is introduced in an expressible form and has an ability to produce L-lysine or L-arginine; and (3) Producing L-lysine or L-arginine comprising culturing (II) in a medium and collecting L-lysine or L-arginine from the culture.

BIOTECHNOLOGY - Preferred DNA: (I) encodes a mutant protein which substantially consists only of the hydrophobic helixes. Preferably, the mutant protein has all of the six hydrophobic helixes. (I) encodes a peptide containing the first to third hydrophobic helixes from the N-terminus and a peptide containing the fourth to sixth hydrophobic helixes from the N-terminus. Most preferably, (I) encodes LysE protein from a coryneform bacterium. The **methanol** assimilating bacterium as described above is a Methylophilus bacterium.

USE - (I) is used for encoding a protein which facilitates excretion of L-lysine, L-arginine or both of these L-amino acids to outside of a cell of a **methanol** assimilating bacterium when (I) is introduced into the bacterium. (II) is useful for producing L-lysine or L-arginine (claimed).

ADVANTAGE - The promotion of excretion of the L-amino acids from the inside of the cell to the outside to the cell is observed as increased concentrations of the L-amino acids accumulated in the medium during the culture of the **methanol** assimilating bacterium containing (I) compared with the concentrations provided by the **methanol** assimilating bacterium not containing (I). Further, the promotion of excretion of the L-amino acids to outside of a cell may also be observed as decrease of intracellular concentrations of the L-amino acids when (I) is introduced into a **methanol** assimilating bacterium.

EXAMPLE - An lysE gene which was a homologous gene of the gene facilitating excretion of L-lysine known for corynebacterium bacteria, was cloned from Brevibacterium bacterium. Construction of pRSlysE was carried out as follows. In order to introduce lysE into a Methylophilus bacterium a known plasmid pRS International Patent Publication in Japanese (Kohyo) No.3-501682 was used to construct a plasmid pRSlysE for expression of lysE. pRS is a plasmid having the vector segment of the pVIC40 plasmid International Patent Publication WO90/04636, International Patent Publication in Japanese No.3-501682 and obtained from pVIC40 by deleting a DNA region encoding the threonine operon contained in the plasmid. The plasmid pVIC40 was derived from a broad host spectrum vector plasmid pAYC32 Chistorerdov, A.Y., Tsygankov, Y.D., Plasmid, 1986, 16, 161-167 which is a derivative of RSF1010. First, a plasmid pRStac having the tac promoter was constructed from pRS. The pRStac plasmid was constructed as follows. The pRS vector was digested with restriction enzymes EcoRI and PstI and added with a phenol/chloroform solution and

mixed with it to terminate with the reaction. After the reaction mixture was centrifuged, the upper layer was collected and DNA's were collected by ethanol precipitation and separated. A DNA fragment of 8 kbp was collected, the tac promoter region was amplified by polymerase chain reaction (PCR) using the pKK223-3 plasmid (expression vector) as a template. The DNA fragment containing the amplified tac promoter was purified and then digested at EcoRI and EcoT221 sites. Then, the reaction mixture was added with a phenol/chloroform solution and mixed after the reaction mixture was centrifuged the upper layer was collected and DNA's were collected by ethanol precipitation and separated. A DNA fragment of about 0.15 kbp was collected. The digestion product of pRS vector and the tac promoter region fragment prepared as described above were ligated. The ligation reaction solution was used to transform Escherichia coli JM109 competent cells. The cells were plated on LB agar medium containing 20 mg/l of streptomycin and incubated overnight at 37 degreesC. The colonies appeared on the agar medium were each inoculated into LB liquid medium containing 20 mg/l of streptomycin and cultured at 37 degreesC for 8 hours with shaking. Plasmid DNA was extracted from each culture and structure of each plasmid was confirmed by digestion with restriction enzymes to obtain pRStac. pRStac obtained as described above was digested with Sse83871 and SapI added with a phenol/chloroform solution and mixed with it to terminate the reaction. After the reaction mixture was centrifuged, the upper layer was collected and DNA's were collected by ethanol precipitation and separated on 0.8% agarose gel to obtain a DNA fragment of about 9.0 kbp. lysE gene fragment was also amplified by PCR using chromosome extracted from the Brevibacterium lactofermentum 2256 strain as a template. The obtained fragment was purified and then digested with Sse83871 and SapI. The reaction mixture was added with a phenol/chloroform solution and mixed with it to terminate the reaction. After the reaction mixture was centrifuged, the upper layer was collected and DNA's were collected by ethanol precipitation and further collected from 0.8% agarose gel. The digestion product of the pRStac vector and the lysE gene region fragment prepared as described above were ligated. This ligation reaction solution was used to transform E.coli JM109 competent cells. The cells were plated on LB agar medium containing 20 mg/l of streptomycin and incubated overnight at 37 degreesC. The colonies appeared on the agar medium were each inoculated into LB liquid medium containing 20 mg/l of streptomycin and cultured at 37 degreesC for 8 hours with shaking. Plasmid DNA was extracted from each culture broth by the alkali-sodium dodecyl sulfate (SDS) method and structure of each plasmid was confirmed by digestion with restriction enzymes and determination of nucleotide sequence to obtain pRSlysE. pRSdapA obtained as described above was introduced into Methylophilus methylotrophus strain. The obtained transformant was referred to as AS1/pRSdapA. Methylophilus methylotrophus AS1 strain introduced with pRSlysE24 was referred to as AS1/pRSlysE24 and M.methylotrophus AS1 strain introduced with the pRS plasmid was referred to as AS1/pRS. The intracellular **L-amino acid** concentration and the **L-amino acid** concentration in culture supernatant of above strains were determined. With AS1/pRSlysE24, L-lysine accumulation substantially equivalent to that of AS1/pRSdapA was observed in the medium. On the other hand, with AS1/pRSlysE24, the intracellular L-lysine concentration was suppressed to a low level, and it was considered that L-lysine was excreted to outside of the cells due to the introduction of the lysE24 gene. Further, concentrations of other L-amino acids in the culture supernatant were also investigated. As a result, it was found that L-arginine accumulated in AS1/pRSlysE24. Thus, it was found that lysE24 had excretion activity not only for L-lysine but also for L-arginine. (23 pages)

ACCESSION NUMBER: 2003-11377 BIOTECHDS

TITLE: Novel DNA encoding variant of LysE protein from a coryneform bacterium, when introduced into **methanol** assimilating bacterium, facilitates excretion of L-lysine

and/or L-arginine to outside of a cell;
plasmid pRSlysE-mediated lysE gene transfer to Methylophilus sp. for amino acid **production**

AUTHOR: GUNJI Y; YASUEDA H
PATENT ASSIGNEE: AJINOMOTO CO INC
PATENT INFO: EP 1266966 18 Dec 2002
APPLICATION INFO: EP 2002-12539 5 Jun 2002
PRIORITY INFO: JP 2001-177075 12 Jun 2001; JP 2001-177075 12 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-241171 [24]

L4 ANSWER 11 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Novel nitrilase polypeptide, useful for making (R)- or (S)-ethyl-4-cyano-3-hydroxybutyric acid or (R)- or (S)-mandelic acid or (S)- or (R)-phenyl lactic acid derivative and for producing pharmaceutical composition, and food additive;
vector-mediated recombinant protein gene transfer and expression in host cell for use in pharmaceutical and food industry and peptidomics

AN 2003-10320 BIOTECHDS

AB DERWENT ABSTRACT:
NOVELTY - An isolated polypeptide (I) comprising consecutive amino acids having a sequence at least 50% identical to any one of the 192 (300-400 residues long) amino acid sequences (S1) as given in the specification, and having nitrilase activity, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) comprising consecutive nucleotides having a sequence at least 50% identical to any one of 192 (900-1100 base pairs long) sequences (S2) all given in the specification, or having a sequence at least 79% identical to a fully defined sequence (S3) of 1026 nucleotides as given in the specification, where the nucleic acid encodes a polypeptide having nitrilase activity; (2) a fragment of (II) encoding a polypeptide having nitrilase activity; (3) an isolated nucleic acid complementary to (II); (4) an isolated nucleic acid that hybridizes to (II) under stringent conditions; (5) a nucleic acid probe (III) comprising about 15-50 nucleotides, where at least 15 contiguous nucleotides are at least 50% complementary to a nucleic acid target region within (S2); (6) a nucleic acid vector (IV) capable of replication in a host cell, comprising (II) or (III); (7) a host cell (V) comprising (II) or (III); (8) a host organism (VI) comprising (V); (9) an isolated nucleic acid (VII) encoding a polypeptide comprising consecutive amino acids having a sequence at least 50% identical to (S1), where the polypeptide has nitrilase activity; (10) an isolated nucleic acid (VIII) encoding a polypeptide comprising at least 10 consecutive amino acids having a sequence identical to a portion of (S1); (11) a fragment of (I) which is at least 20 amino acids in length, and has nitrilase activity; (12) a peptidomimetic (IX) of (I) or its fragment having nitrilase activity; (13) a codon-optimized polypeptide of (I) or its fragment, having nitrilase activity, where the codon usage is optimized for a particular organism or cell; (14) a purified antibody (X) that specifically binds to (I) or its fragment having nitrilase activity; (15) a fragment of (X) which specifically binds to (I); (16) a liquid or dry enzyme preparation (XI) which comprises (I); (17) a composition comprising (I), (II), (III), (VII) or (IX), having nitrilase activity or their combination; (18) making (I); (19) a computer readable medium which stores (S1) or (S2); (20) a computer system comprising a processor and a data storage device which stores (S1) and/or (S2); (21) identifying a feature in a sequence which comprises (S1), (S2) or (S3), involves inputting the sequence into computer, running a sequence feature identification program on the computer, and identifying the feature in the sequence; (22) assay for identifying functional variant of polypeptide, involves obtaining the variant of (I), contacting the variant with the substrate having cyanohydrine or aminonitrile moiety,

measuring amount of reaction product produced by each of the variant, and identifying the variant which is capable of producing nitrilase reaction product; (23) an isolated polypeptide exhibiting nitrilase activity, and comprising three consensus subsequences, such as FPETF, RRKLXPT and LXCWEHXXP, respectively; and (24) an isolated polypeptide exhibiting nitrilase activity, comprising three consensus subsequences, such as FPEXX, XRKLXPT and LXCWEXXXP, respectively; (25) a method for making (R)- or (S)-ethyl-4-cyano-3- hydroxybutyric acid; (26) a method for making an (R)-mandelic acid comprising (R)-2-chloromandelic acid; (27) a method for making (S)-mandelic acid comprising (S)-methyl benzyl cyanide; (28) a method for making (S)- or (R)-phenyl lactic acid derivative, by admixing phenyllactocyanonitrile with (I), its fragment or (IX); (29) a method for generating a nucleic acid variant encoding a polypeptide having nitrilase activity, having an altered biological activity from that which naturally polypeptide, which involves modifying (II) or (III) by substitution, deletion or addition mutations;

BIOTECHNOLOGY - Preparation: (I) is prepared by introducing a nucleic acid encoding the polypeptide in a host cell under conditions that permit **production** of the polypeptide by the host cell, and recovering the polypeptide so produced (claimed). Preferred Polypeptide: (I) comprises consecutive amino acids having preferably 98% identity to (S1). Most preferably, (I) comprises the sequence of (S1). (I) or its fragment of (IX) is affixed to a solid support such as a gel, resin, polymer, ceramic, glass, microelectrode or their combinations. Preferred Nucleic Acid: (II) comprises consecutive nucleotides having at least 96%-100% identical to (S2) or (S3), or is substantially identical to (S2), or has a sequence identical to (S2). In (III), the 50 consecutive nucleotides are preferably 98-100% complementary to the nucleic acid target region. The nucleic acid consists essentially of 20-50 nucleotides. (VII) encodes a polypeptide comprising consecutive amino acids having a sequence which preferably 95-100% identity to (S1), or comprises consecutive amino acids having the sequence of (S1). Most preferably, (VII) encodes a polypeptide comprising at least 10 consecutive amino acids having a sequence identical to a portion of (S1). (II), (III) or (VII) is affixed to a solid support. Preferred Organism: (VI) comprises a gram negative bacteria (e.g., Escherichia coli or Pseudomonas fluorescens), gram positive bacteria (e.g., Streptomyces diversa, Lactobacillus gasseri, Lactococcus lactis, Lactococcus cremoris or Bacillus subtilis) or an eukaryotic organism e.g., Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Kluyveromyces lactis, Hansenula polymorpha or Aspergillus niger. Preferred Preparation: (XI) is affixed to a solid support. Preferred Method: The method of (29) further comprises assaying the polypeptides encoded by the modified nucleic acids for altered nitrilase activity. The modifications are made by polymerase chain reaction (PCR), error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, etc.

USE - (I) or its fragment or (IX) is useful for hydrolyzing a nitrile to a carboxylic acid under aqueous conditions of pH 8 and/or at 37-45 degrees Centigrade; hydrolyzing a cyanohydrin moiety or an aminonitrile moiety of a molecule; making a chiral alpha-hydroxy acid molecule or a chiral amino acid molecule; and making a composition or its intermediate, which involves admixing a precursor (comprising a cyanohydrin moiety or an aminonitrile moiety) of the composition or its intermediate, with (I), or its fragment or (IX), such that the cyanohydrin or aminonitrile moiety is hydrolyzed thereby making the composition or its intermediate. The composition or its intermediate preferably comprises (S)-2-amino-4-phenyl butanoic acid or an **L -amino acid**. The method is also useful for making a composition comprising a food additive or a pharmaceutical drug. (I) is useful for making (R)- or (S)-ethyl-4-cyano-3- hydroxybutyric acid. (I) is also useful for making an (R)-mandelic acid comprising (R)-2-chloromandelic acid, for making (S)-mandelic acid comprising

(S)-methyl benzyl cyanide, or for making (S)- or (R)-phenyl lactic acid derivative. (II) or (III) is useful for generating a nucleic acid variant encoding a polypeptide having nitrilase activity, or having an altered biological activity from that which naturally polypeptide. (II), (III) or (VII) is useful for making a polynucleotide from two or more nucleic acids, by identifying regions of identity and regions of diversity between the two or more nucleic acids, providing a set of oligonucleotides which correspond in sequence to at least two of the two or more nucleic acids, and extending the oligonucleotides with a polymerase, to make the polynucleotide. (I), (II), (III) or (VII) is useful for screening assay for identifying nitrilase activity. (I) or its fragment or (IX) is useful in an industrial process for producing a pharmaceutical composition, food additive, for catalyzing the breakdown of waste, producing a detergent, producing a food product, or producing a drug intermediate which involves use of the polypeptide to hydrolyze a hydroxyglutarylnitrile substrate. Preferably, the drug intermediate **production** process is the process for the **production** of LIPITOR. (I) or its fragment or (IX) is also useful for modifying a molecule and for identifying a modified compound. Fragment of (I) is useful for identifying a functional fragment or polypeptide, which is capable of producing a nitrilase reaction product. (II) or (III) is useful for preparing a transgenic organism (all claimed).

ADVANTAGE - Using (I), dynamic resolution conditions can be established due to racemization of substrate under aqueous conditions. (I) has increased activity and stability at increased pH and temperature.

EXAMPLE - For each library to be screened for nitrilases, an infection was set up as follows. 5 ml of an OD(600)nm = 1 resuspension of SEL700 cells and 1 ml of the phagemid library to be screened were combined. The combination was incubated in a 37 degrees Centigrade waterbath for 45 min. Using the infection, serial dilutions were made in 10 mM MgSO4, using 10 microlitres aliquots of the infection. 60 microlitres of the following dilutions (10 (to the power of -1) dilutions for 10 (to the power of 5) colony forming unit (cfu)/ml of library), (10 (to the power of -1), 10 (to the power of -2) dilutions for 10 (to the power of 6) cfu/ml of library), and (10 (to the power of -2) and 10 (to the power of -3) dilutions for 10 (to the power of 7) cfu/ml of library). The cells in the infection were centrifuged. The supernatant was decanted from the resulting pellets. The cells were resuspended in residual liquid. All of the resuspended cells were deposited onto a single large LB-Kan50 plate. All plates were incubated at 30 degrees Centigrade overnight. The cells of each infection plate were resuspended with 4 mls 10 mM MgSO4. The resuspensions were placed in a tube. The remaining cells on each plate were resuspended with 3 mls 10 mM MgSO4 and combined with the first resuspension from the same plate. The volume of each tube was brought to 12 ml with 10 mM MgSO4, the tubes were vortexed. The tubes were centrifuged. The supernatant was decanted from each resuspension. The washed cells in each tube were resuspended with 10 ml 20 mM MgSO4. For each resuspension, selection cultures were set up using the following processes. The nitrilase selection medium was prepared, using 1 x M9 medium with 0.2% glucose, no nitrogen and 50 micrograms/ml kanamycin, 5 ml of the medium was aliquoted into a 50 ml screw top conical tube, 25 microlitres of the stored resuspension was added to the tube, 5 microlitres adiponitrile was added to the tube, to bring the final concentration to 8.8 mM. Additional nitrile substrates may be used, in place of adiponitrile. Ten (10) microlitres of selection culture with growth was streaked out onto a small LB-kan50 plate and allowed to grow for 2 nights at 30 degrees Centigrade. Five isolated cfu were picked and each was grown in 2 ml nitrilase selection medium at 30 degrees Centigrade. Each culture was monitored and was removed. One (1) ml of culture was used to do a plasmid preparation and was eluted with 40 microlitres elution buffer. Five to eight (5-8) microlitres DNA was cut with PstI/XhoI or SacI/KpnI restriction enzymes to remove insert from vector. A restriction fragment length polymorphism (RFLP) determination

was carried out to identify the size of the insert. The insert was sequenced. Nitrilases of the invention were screened against target substrates. Of those showing hydrolytic activity in primary screen, enzymes with enantioselectivities above 20% enantiomeric excess (ee) were selected for further characterization. Those enzymes were selected base on, having activity against one of the substrates of interest and exhibition of greater than 35% ee (enantiomeric excess). The products used for screening were, D-Phenylglycine, L-Phenyllactic acid, (R) 2-chloromandelic acid, (S)-Cyclohexylmandelic acid, L-2-methylphenylglycine, (S)-2-amino-6-hydroxy hexanoic acid, and 4-methyl-L-leucine. Of the enzymes identified active on phenylglycinonitrile, the enantioselectivity of several enzymes was shown to remain above the success criterion of 35% ee. The preliminary characterization data indicated that some of the enzymes exhibited high enantioselectivities for D-phenylglycine, with corresponding conversion to product of 40-60%. From screening of the nitrilases, against target substrate (R)-2-chloromandelic acid, it was found that higher temperatures and neutral pH appeared to lead to the highest activity for the active enzymes. For the majority of the nitrilases, the enantioselectivity also increased at higher temperatures, particularly 38 degrees Centigrade. The enzymes retained their activity in the presence of up to 25% **methanol** or 10% isopropanol, in many of these cases, the enantioselectivity was also enhanced. Activity in biphasic systems was largely comparable to aqueous conditions, particularly with hexane as the non-aqueous phase, varying tolerances to toluene were observed between the different nitrilases.(560 pages)

ACCESSION NUMBER: 2003-10320 BIOTECHDS
TITLE: Novel nitrilase polypeptide, useful for making (R)- or (S)-ethyl-4-cyano-3-hydroxybutyric acid or (R)- or (S)-mandelic acid or (S)- or (R)-phenyl lactic acid derivative and for producing pharmaceutical composition, and food additive;
vector-mediated recombinant protein gene transfer and expression in host cell for use in pharmaceutical and food industry and peptidomics
AUTHOR: MADDEN M; DESANTIS G; CHAPLIN J A; WEINER D P; MILAN A; CHI E; SHORT J M; BURK M
PATENT ASSIGNEE: DIVERSA CORP; MADDEN D
PATENT INFO: WO 2003000840 3 Jan 2003
APPLICATION INFO: WO 2002-US15983 15 May 2002
PRIORITY INFO: US 2002-351336 22 Jan 2002; US 2001-300189 21 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-201417 [19]

L4 ANSWER 12 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Efficient **production** of L-amino acids e.g. L-lysine by fermenting transformed Methylopillus bacterium with enhanced dihydrodipicolinate-synthase and aspartokinase activities or casamino acid requirement using **methanol**;
plasmid RSF and plasmid pRS-lysC80-mediated aspartate-kinase, aspartic semi-aldehyde-dehydrogenase, dihydropicolinate-reductase or diaminopimelate decarboxylase gene transfer
AN 2001-02341 BIOTECHDS
AB A bacterium of Methylophilus sp., preferably Methylophilus methylotrophus, can produce an **L-amino acid** efficiently, which is a transformed bacterium with enhanced enzyme activity, is new. Also claimed are: a process for preparing an **L -amino acid**; a DNA having a 1,981 bp sequence encoding a 409 amino acid protein sequence having aspartate-kinase (EC-2.7.2.4) activity; a DNA having a 1,452 bp sequence encoding a 370 amino acid protein sequence with aspartic semi-aldehyde-dehydrogenase activity; a DNA having a 3,098 bp sequence encoding a 296 amino acid

protein sequence with dihydropicolinate-synthase (EC-4.2.1.52) activity; a DNA having a 3,390 bp sequence encoding a 286 amino acid protein sequence with dihydropicolinate-reductase activity; and a DNA having a 2,566 bp sequence encoding the 415 amino acid protein sequence with diaminopimelate-decarboxylase (EC-4.1.1.20) activity. The method is for the **production** of L-amino acids including L-lysine, L-valine, L-leucine, L-isoleucine or L-threonine. In an example, a Methylophilus sp. was transformed with plasmid RSF containing mutated dapA and expression plasmid pRS-lysC80. (92pp)

ACCESSION NUMBER: 2001-02341 BIOTECHDS
TITLE: Efficient **production** of L-amino acids e.g. L-lysine by fermenting transformed Methylopillus bacterium with enhanced dihydrodipicolinate-synthase and aspartokinase activities or casamino acid requirement using **methanol**;
plasmid RSF and plasmid pRS-lysC80-mediated aspartate-kinase, aspartic semi-aldehyde-dehydrogenase, dihydropicolinate-reductase or diaminopimelate decarboxylase gene transfer
AUTHOR: Gunji Y; Yasueda H; Sugimoto S; Tsujimoto N; Shimaoka M; Miyata Y; Oba M
PATENT ASSIGNEE: Ajinomoto
LOCATION: Tokyo, Japan.
PATENT INFO: WO 2000061723 19 Oct 2000
APPLICATION INFO: WO 2000-JP2295 7 Apr 2000
PRIORITY INFO: JP 1999-368097 24 Dec 1999; JP 1999-103143 9 Apr 1999
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2000-672679 [65]

L4 ANSWER 13 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI New (4S)-hydroxy-3-methyl-2-ketopentanoic acids;
stereospecific hydroxymethylketopentanoic acid **production** by **L-amino-acid**-oxidase or Morganella morganii for use as a flavor
AN 1995-01122 BIOTECHDS
AB 4S-hydroxy-3-methyl-2-ketopentanoic acid of formula (I) is new. Also new is **production** of stereospecific 5S-3-hydroxy-4,5-dimethyl-2(5H)-furanone of formula (III), which involves lactonizing (I). (I) is produced by reacting L-4-hydroxyisoleucine (II) with a microorganism with **L-amino-acid**-oxidase (EC-1.4.3.2) activity or with the enzyme alone. (I) is useful as a flavor precursor, while (III) is a flavor. In an example, Morganella morganii DSM 30117 was grown in a fermentor for 20 hr at 30 deg and pH 7 in culture medium containing 10 g/l glucose, 10 g/l casein hydrolyzate and salts. The cells were centrifuged, washed with 0.02 M sodium phosphate buffer (pH 7) and the cell solution was reacted with 7.7 mmol L-4-hydroxyisoleucine (prepared from 4 kg seeds of Trigonella foenum graecum L.) and the mixture was incubated at 30 deg for 6 hr with agitation. The cells were separated from the mixture by centrifugation and the residue was lyophilized. The residue was extracted with **methanol** and 2-keto-3-methyl-4-hydroxyvaleric acid sodium salt was prepared by adding methyl-tert. butyl ether. (10pp)

ACCESSION NUMBER: 1995-01122 BIOTECHDS
TITLE: New (4S)-hydroxy-3-methyl-2-ketopentanoic acids;
stereospecific hydroxymethylketopentanoic acid **production** by **L-amino-acid**-oxidase or Morganella morganii for use as a flavor
AUTHOR: Lerch K
PATENT ASSIGNEE: Givaudan-Roure
PATENT INFO: EP 623580 9 Nov 1994
APPLICATION INFO: EP 1994-106577 27 Apr 1994

PRIORITY INFO: CH 1993-1394 6 May 1993
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1994-343246 [43]

L4 ANSWER 14 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI **L-amino acid production** e.g.
L-threonine, L-lysine, L-valine, L-alanine, L-aspartic acid, L-glutamic acid, L-isoleucine, L-leucine;
by beta-fluoropyruvic acid- 3-bromopyruvic acid-resistant Methylobacillus sp. using a **methanol** C-source
AN 1994-11177 BIOTECHDS
AB **L-amino acid** is prepared by culturing Methylobacillus sp. (which is resistant to halogenated pyruvic acid) and collecting the amino acid from the culture medium. Preferably: (1) the halogenated pyruvate is beta-fluoropyruvic acid or 3-bromopyruvic acid; (2) the medium contains **methanol** as a principle C-source; and (3) the **L-amino acid** is L-threonine, L-lysine, L-valine, L-alanine, L-aspartic acid, L-glutamic acid, L-isoleucine or L-leucine. This method allows efficient **production** of an **L-amino acid** from a cheap **methanol** C-source. (6pp)
ACCESSION NUMBER: 1994-11177 BIOTECHDS
TITLE: **L-amino acid production**
e.g. L-threonine, L-lysine, L-valine, L-alanine, L-aspartic acid, L-glutamic acid, L-isoleucine, L-leucine;
by beta-fluoropyruvic acid- 3-bromopyruvic acid-resistant Methylobacillus sp. using a **methanol** C-source
PATENT ASSIGNEE: Kyowa-Hakko
PATENT INFO: JP 06133788 17 May 1994
APPLICATION INFO: JP 1992-291140 29 Oct 1992
PRIORITY INFO: JP 1992-291140 29 Oct 1992
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1994-196169 [24]

L4 ANSWER 15 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI **Production** of stereospecific N-acyl amino acid;
using Aspergillus and Penicillium aminoacylase
AN 1990-08405 BIOTECHDS
AB An acyl donor and L-aminoacylase (EC-3.5.1.14) are mixed to form a racemic solution, which acylates **L-amino acid** in the presence of an organic solvent at 35-40 deg and pH 6-8 to form N-acyl-**L-amino acid**. The method is simple and gives good yields of high purity products. The acyl donor is preferably an acetate or propionic acid salt. The organic solvent is preferably **methanol**, ethanol, dimethyl sulfoxide, acetone, dioxane, glycerine, ethylene glycol, isopropanol, acetonitrile or tetrahydrofuran. The concentration of the organic solvent is preferably 10-90%, especially 20-80%. The aminoacylase is preferably derived from an Aspergillus or a Penicillium sp. (4pp)
ACCESSION NUMBER: 1990-08405 BIOTECHDS
TITLE: **Production** of stereospecific N-acyl amino acid;
using Aspergillus and Penicillium aminoacylase
PATENT ASSIGNEE: Amano-Pharm.
PATENT INFO: JP 02072897 13 Mar 1990
APPLICATION INFO: JP 1988-224088 7 Sep 1988
PRIORITY INFO: JP 1988-224088 7 Sep 1988
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1990-121058 [16]

L4 ANSWER 16 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI   Microbial and enzymatic processes for the **production** of
        biologically active amino acids;
            L-amino acid and D-amino acid
          production (conference paper)
AN   1989-09694 BIOTECHDS
AB   Tyrosine-phenol-lyase (EC-4.1.99.2), tryptophanase (EC-4.1.99.1), cystathionine-gamma-lyase (EC-4.4.1.1), O-acetyl-L-serine-sulfhydrase and beta-chloro-D-alanine-dehydrochlorinase catalyzed the synthesis of L-tyrosine, L-tryptophan, L-cysteine, D-cysteine and related amino acids in significantly high yields. Cystathionine-gamma-lyase and O-succinylhomoserine-thiol-lyase (EC-4.2.99.9) catalyzed the synthesis of cystathionine and related amino acids in yields of nearly 100% under suitable reaction conditions. L-serine was synthesized from glycine and **methanol** by **methanol**-dehydrogenase and serine-hydroxymethyltransferase. Hyphomicrobium methylovorum strain KM 146 was selected as a serine producer. Under optimum reaction conditions, L-serine was produced in a 20% yield from 100 mg/ml glycine. A glycine-resistant mutant showed 1.5-fold productivity higher than the parent. A new process was developed for the **production** of D-p-hydroxyphenylglycine, an important component of semisynthetic penicillins and cephalosporins. Dihydropyrimidinase was used and the process was the most economical for industrial **production**. (12 ref)

ACCESSION NUMBER: 1989-09694 BIOTECHDS
TITLE: Microbial and enzymatic processes for the **production** of biologically active amino acids;
    L-amino acid and D-amino
    acid **production** (conference paper)
AUTHOR: Yamada H
LOCATION: Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan.
SOURCE: Eur.Congr.Biotechnol.; (1987) Vol.4, 689-92
DOCUMENT TYPE: Journal
LANGUAGE: English

L4   ANSWER 17 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI   Microbial and enzymatic process for the **production** of optically
        active amino acids;
          amino acid preparation using enzyme (conference abstract)
AN   1989-06906 BIOTECHDS
AB   Stereospecific amino acid preparation using enzymes was studied. Tyrosine-phenol-lyase (EC-4.1.99.2), tryptophanase (EC-4.1.99.1), cystathionine-gamma-lyase (EC-4.4.1.1), O-acetylserine-thiol-lyase (EC-4.2.99.8) and 3-chloro-D-alanine-dehydrochlorinase (EC-4.5.1.2) were used to synthesize aromatic and sulfur-containing amino acids through beta-replacement and the reverse of alpha, beta-elimination reactions. Cystathionine-gamma-lyase and O-succinylhomoserine-thiol-lyase (EC-4.2.99.9) were used to synthesize cystathionine and related amino acids from L-acyl-L-homoserine and cysteine analogs, in almost 100% yields. L-serine was synthesized from glycine and **methanol** by phosphoribosylglycinamide-formyltransferase (EC-2.1.2.2) of methylotrophic bacteria. For synthesis of D-phenylglycine and related D-amino acids, dihydropyrimidinase (EC-3.5.2.2) catalyzed ring-opening of dihydropyrimidines, and hydrolyzed 5-monosubstituted hydantoins. Hydantoins were hydrolyzed to D-forms of the N-carbamoyl glycine derivatives. These N-carbamyl amino acids were transformed to D-amino acids by treatment with NaNO2 under acidic conditions. (0 ref)

ACCESSION NUMBER: 1989-06906 BIOTECHDS
TITLE: Microbial and enzymatic process for the **production** of optically active amino acids;
    amino acid preparation using enzyme (conference abstract)
AUTHOR: Yamada H
LOCATION: Department of Agricultural Chemistry, Kyoto University, Kyoto

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                      606, Japan.
SOURCE:               Eur.Congr.Biotechnol.; (1987) Vol.2, 19
DOCUMENT TYPE:        Journal
LANGUAGE:             English

L4     ANSWER 18 OF 18  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI     Racemization of amino acid esters by aromatic aldehydes in basic
       non-aqueous solvents;
          potential use with hydrolase for the stereospecific production
          of L-amino acids
AN     1985-03652  BIOTECHDS
AB     The racemization of optically active leucine methyl ester in the presence
       of pyridoxal or pyridoxal 5-phosphate was studied in water at neutral pH
       and in methanol at different concentrations of triethylamine.
       In water, the racemization rate with pyridoxal 5-phosphate was 10 times
       faster than with pyridoxal.  In methanol containing
       triethylamine, the effectiveness of pyridoxal and pyridoxal 5-phosphate
       were of the same order and both were more effective than in water.
       Racemization of amino acid esters by combined pyridoxal and triethylamine
       catalysis occurred in diverse organic media such as monohydric alcohols
       and halogenated hydrocarbons.  Both the aromatic ring N and the hydroxyl
       group at position 2 (as in pyridoxal) were important for high catalytic
       properties in the aromatic aldehydes.  Pyridoxal was immobilized by
       covalent linking to an insoluble polyaryldiazonium reagent and suspended
       in basic methanol solutions of amino acid ester. Racemization
       took place in the heterogeneous system.  The solid catalyst for
       racemization will be used in combination with a chiral-specific
       hydrolase, also insolubilized, for the stereospecific production
       of L-amino acids.  (5 ref)
ACCESSION NUMBER: 1985-03652  BIOTECHDS
TITLE:                Racemization of amino acid esters by aromatic aldehydes in
                      basic non-aqueous solvents;
                         potential use with hydrolase for the stereospecific
                         production of L-amino acids
AUTHOR:               Pugniere M; San Juan S; *Previero A
LOCATION:             INSERM, 60 rue de Navacelles, 34100 Montpellier, France.
SOURCE:               Biotechnol.Lett.; (1985) 7, 1, 31-36
                      CODEN: BILED3
DOCUMENT TYPE:        Journal
LANGUAGE:             English


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L4            18 S L2 AND METHANOL
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L5             0 L4 AND (6-PHOSPHOGLUCONATE DEHYDRATASE)
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Terms	Documents
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	DB=USPT; PLUR=YES; OP=OR		
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L6	gunji.in.	229	L6
L5	L4 and methanol	114029	L5
L4	L3 and l1	288018	L4
L3	L-amino acid production	1165210	L3
L2	(6-phosphogluconate dehydratase activity)	403367	L2
L1	(6-phosphogluconate dehydratase activity)	403367	L1

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IBM Technical Disclosure Bulletins

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L6	gunji.in.	229	L6
L5	L4 and methanol	114029	L5
L4	L3 and l1	288018	L4
L3	L-amino acid production	1165210	L3
L2	(6-phosphogluconate dehydratase activity)	403367	L2
L1	(6-phosphogluconate dehydratase activity)	403367	L1

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1. Document ID: US 6177541 B1

L7: Entry 1 of 2 File: USPT Jan 23, 2001

US-PAT-NO: 6177541
DOCUMENT-IDENTIFIER: US 6177541 B1

TITLE: Process for producing an isocyanurate derivative

DATE-ISSUED: January 23, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ikeda; Hisao	Funabashi			JP
Koda; Toshinari	Funabashi			JP
Gunji; Yasuhiro	Funabashi			JP

US-CL-CURRENT: 528/487; 528/287, 528/422, 528/423, 528/503, 528/72, 528/80

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

2. Document ID: US 5871843 A

L7: Entry 2 of 2 File: USPT Feb 16, 1999

US-PAT-NO: 5871843
DOCUMENT-IDENTIFIER: US 5871843 A

TITLE: Laminate and process for its production

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yoneda; Takashige	Yokohama			JP
Fukawa; Makoto	Yokohama			JP
Morimoto; Takeshi	Yokohama			JP
Sato; Kazuo	Yokohama			JP
Gunji; Fumiaki	Yokohama			JP
Nishimura; Hiromichi	Yokohama			JP
Takeda; Satoshi	Yokohama			JP
Hayashi; Yasuo	Yokohama			JP
Fujita; Hiroyuki	Kanagawa			JP

US-CL-CURRENT: 428/337; 427/165, 428/315.5, 428/428

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

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